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Research Article

Maturation and Developmental Competence of Selectively Enucleated Germinal Vesicle Oocytes of Mammals upon nuclear transfer

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Abstract

Interaction between the donor nuclei and the recipient cytoplasts is considered as one of the fundamental questions in the field of assisted reproductive technology. The resulting artificial oocytes might be used for embryonic/somatic cloning or treatment of infertility. Because of difficulties in co-operation between donor nuclei (embryonic & somatic) and recipient cytoplasts (Metaphase II), trials were carried out to investigate maturation and developmental competence of fully grown germinal vesicle (GV) cytoplasts upon reconstitution with germ or embryonic or somatic nuclei. In order to obtain the recipient cytoplasts, fully grown germinal vesicle (GV) oocytes were completely or selectively enucleated. For complete enucleation of GV oocyte, the whole nuclei were removed, and for selective enucleation of GV oocyte, nuclear sap and nucleoli were leaked into the cytoplasts and the nuclear envelope with the attached chromatin was removed. Reconstruction of complete or selective GV cytoplasts with germ or embryonic or somatic donor nuclei at different stages of cell cycle was done to investigate maturation and further developmental competence in some studies. These studies indicated that maturation and developmental competence were affected by the recipient cytoplasts and donor nuclei. Although the embryonic or somatic nuclei were abnormal in their maturation and further development, however, the germ cells were able to mature and develop to offspring. Selective GV cytoplasts enhanced proper pronuclei formation and further developmental competence. Several studies showed that germinal vesicle materials are essential for remodeling the introduced somatic nuclei into proper pronuclei and their further developmental competence. Furthermore, the cumulus cells and the nucleolus are the factors compromised the developmental competence of the reconstituted oocytes. In this article, manipulations of GV oocytes in addition to their maturation and developmental competency upon nuclear transfer were addressed. Moreover, the effects of GV materials, nucleoli and cumulus cells on maturation and developmental competence of reconstituted GV cytoplasts upon nuclear transfer were discussed.

Keywords: *Maturation; selective enucleation, nucleolus*

1. Introduction

Recent interest has grown in the use of germinal vesicle cytoplasts as recipients' of germ or embryonic or somatic nuclei. The resulting reconstructed oocytes seem to be an interesting model for studying the mechanisms of meiotic maturation and developmental potential (**Mohammed *et al.*, 2008; Mohammed 2009 a; Mohammed et al., 2010; Mohammed, 2013, submitted**). Furthermore, the future applying of these artificial gametes for treatment of reproductive disorders or embryonic and somatic cloning can not be excluded.

Advancing maternal age presents a clear inverse relationship with fertility **(Tietze, 1957)** where the risk of conceiving an aneuploid fetus during in-vitro fertilization increases with age **(Hassold and Chiu, 1985).** On the other hand, developmental competence of oocytes collected from young animals was lower than those collected from the adult ones. Transfer nuclei of non-growing, growing or ageing GV oocytes into the fully grown GV ooplast might rescue developmental incompetency ooplasmic or nuclear in nature. The complete fully grown GV cytoplasts could not rescue ageing-associated chromosome misalignment in meiosis upon transfer of ageing GV nuclei from the ageing oocytes **(Cui *et al.*, 2005)** or could not support full term development upon transfer of growing GV nuclei from the growing bovine oocytes (**Bao *et al.*, 2003).** Selective GV cytoplasts may lead to proper maturation of the disabled oocytes and formation of proper "pronuclei" and probably further preimplantation and postimplantation development upon nuclear transfer.

Transfer of embryonic or somatic nuclei into selective GV cytoplasts might enable creating the new type of oocytes carrying the haploid introduced nuclear genome through meiotic maturation. Such artificial gametes could subsequently be fertilized by spermatozoa or artificially activated (**Mohammed *et al.*, 2008; Mohammed 2009 a,b**). In the case of male infertility, the germ or somatic cells (diploid) could be introduced into selective GV cytoplasts and haploidization would be occurred.

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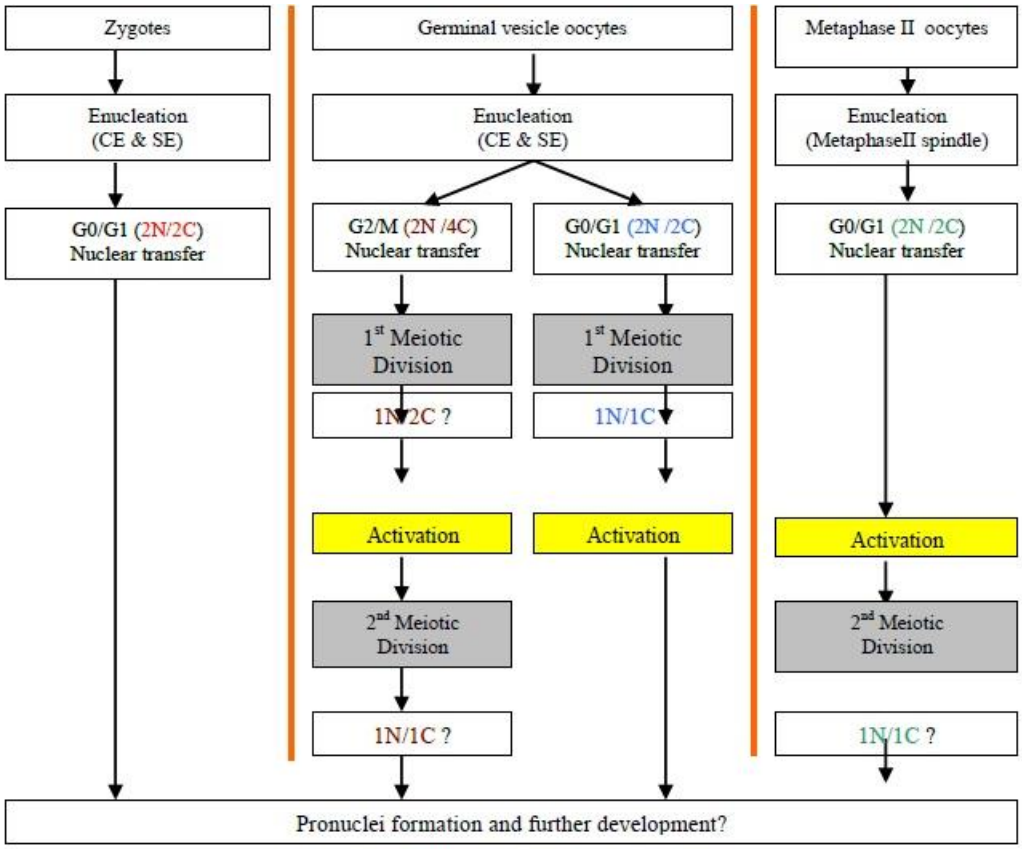

CE; complete enucleation where the whole germinal vesicle nucleus or pronuclei removed.
SE; selective enucleation where the nuclear or pronuclear envelope and chromatin removed only.

Fig. 1. Strategies of reduction divisions of the introduced nuclei (G0/G1 & G2/M) and their further developmental competence into recipient cytoplasts.

Therefore, transfer different nuclei (nuclei of non-growing, growing and ageing GV oocytes, male germ cells, embryonic and somatic cells) into selective cytoplasts may improve or rise developmental competence. Selective enucleation of GV oocytes has provided new alternative of complete enucleation, real or theoretical; to 'generate' gametes from germ or embryonic or somatic cells termed cell haploidization and support propably preimplantation and postimplantation development.

2. Obtaining recipient cytoplasts

The survival of obtaining cytoplasts using enucleation technique is an important step of reconstruction new oocytes in assisted reproductive technology (ART). Enucleation of GV oocytes is difficult due to the large diameter of the GV nucleus and the sensitivity of the plasma membrane. Indirect enucleation method of GV oocytes has been performed previously in rabbit and other mammals **(Sun & Moor, 1991; Meng *et al.*, 1996; Li *et al.*, 2001)** by cutting the zona pellucida and increasing the pressure inside the holding pipette to expel a GV nucleus through the slit in the zona. Improved methods of microsurgical enucleation of GV oocytes have been developed **(Takeuchi *et al.*, 1999; Liu *et al.*, 2000).** However, these methods still involved prior cutting of the zona. These methods of enucleation are time consuming in addition to the adverse effect of preincubation and enucleation solutions and the environmental factors (e.g light and temperature) on maturation and developmental competence of reconstructed oocytes. **Grabarek *et al.*, (2004)** established a direct method (complete enucleation) for microsurgical enucleation of GV oocytes by preincubation and enucleation in the following solution; M2 medium supplemented with 16 mM glucose, 1μg CD, 0.25 μg nocodazole and 0.2 mM dbcAMP. This solution resulted in enlargement of the perivitelline space in GV oocytes, which in turn allowed insertion of the micropipette without the need to produce a slit in the zona pellucida prior to the final stage of micromanipulation. Our protocol (**Mohammed *et al.*, 2008**) in which the GV oocytes were preincubated and enucleated in M2 medium supplemented with 10μg CB, 0.25 μg nocodazole and 0.2 mM dbcAMP resulted in comparable survival rate after enucleation rate and higher survival rate after fusion compared with the previous study. In addition, complete enucleation technique was performed for cumulus-enclosed GV oocytes in the same way as cumulus-free GV oocytes **(Mohammed *et al.*, 2008)** of the GV oocytes with visible GV nucleus.

Selective enucleation (SE) of GV oocytes (**Mohammed *et al.*, 2008**) was performed as selective enucleation of zygotes **Greda *et al.*, (2006)**. Selective enucleation is a technique during it the nuclear sap and the nucleoli leaked into the cytoplasm and the nuclear

envelope with the attached chromatin was removed from the the GV oocytes. Selective enucleation technique was performed the same of cumulus-free and cumulus-enclosed GV oocytes (**Mohammed *et al.*, 2008**) of the GV oocytes with visible GV nucleus.

The GV oocytes with visible GV nucleus such as rabbit, rat, mouse and human oocytes are directly enucleated whereas the GV oocytes with invisible GV nucleus such as bovine, ovine, caprine, buffalo, pig oocytes required ultracentrifugation 10,000-12,000 rpm for 10 minutes firstly to visualize the GV nucleus followed by the complete or selective enucleation.

3. Maturation and further development of the reconstituted oocyte

Meiotic maturation (haploidization) of donor nuclei (germ, embryonic, somatic & stem cells) at different stages of the cell cycle can be achieved by introducing them into recipient cytoplasts that is preprogrammed to undergo meiosis (**Fig. 1**).

3.1. Germ cells

Theoretically, the germ cells can be introduced into MII or GV cytoplasts (**Fig. 1**) for meiotic maturation and further developmental competence. Germinal vesicle transplantation can be accomplished with a high degree of efficiency without compromising the maturation of either the human or mouse oocyte nucleus. Enucleated germinal vesicle (GV) oocytes reconstructed with GV-karyoplasts mature in vitro, yielding as much as 31% of blastocysts after IVF (**Takeuchi *et al.*, 2004**). This developmental potential is comparable to that of intact GV oocytes matured and fertilized in vitro, of which 26% develop into blastocysts (**Chang *et al.*, 2005**). **Kimura and Yanagimachi (1995**) injected nuclei of mouse secondary spermatocytes (1N and 2C) into MII oocytes, whereas nuclei of primary spermatocytes (2N/4C) transferred into GV oocytes in other studies (**Kimura *et al.*, 1998; Ogura *et al.*, 1998; Sasagawa *et al.*, 1998; Nan *et al.*, 2007**), all generating artificially haploidized ‘‘spermatocytes’’ (1N/1C) in vitro that were capable of fertilizing an oocyte in vitro and resulting in full-term development. Likewise, transfer nuclei of non-growing and growing oocytes (2N/4C), collected from newborn and juvenile ovaries and adult ovaries, into recipient cytoplasts of fully grown germinal vesicle (GV) oocytes results in the re-initiation of meiosis in vitro (**Bao *et al.*, 2000; Obata *et al.*, 2000, Bao *et al.*, 2003**). Furthermore, the chromosomes in the first polar body (1N/2C), which is a by-product of an oocyte during meiosis, have the same potential as their sister chromosomes remaining in the oocyte. After injection of the first polar body into an enucleated MII oocyte, a haploid genome (1N/1C) can be generated by the second meiotic spindle of an MII oocyte (**Wakayama and Yanagimachi 1998**). The generation of viable offspring has proven that the creation of artificially halploidized ‘‘oocyte’’ (1N/1C) was also achieved by the machineries of the first and second meiotic spindles, present in the resumption of meiosis-competent oocyte.

3.2. Embryonic and somatic cells

The embryonic and somatic cells at different satges of the cell cycle can be introduced into MII or GV oocytes (Fig 1) for meiotic maturation and further developmental competence. Initial trials to obtain mature oocytes were unsuccessful after replacement of the nucleus of GV oocyte by nucleus from somatic cell (**Kubelka and Moor, 1997; Fulka *et al.*, 2002**). In the previous studies, reconstructed GV oocytes with nuclei from fibroblasts or cumulus cells did not divide during subsequent in vitro maturation. However, recent reports describe the meiotic maturation, although associated with some anomalies, of the enucleated GV oocytes after nuclear transfer of embryonic or somatic cells (**Chang *et al.*, 2004; Grabarek *et al.*, 2004; Polanski *et al.*, 2005; Mohammed *et al.*, 2008**). In those studies, meiotic maturation after embryonic or somatic nuclear transfer was associated with abnormalities in cytokinesis and chromosome condensation as well as premature division. About two-thirds of reconstructed oocytes with embryonic or somatic nuclei were extruded the first polar body. A fraction of polar bodies is of abnormal (large) size. Moreover, aberrant meiotic spindles as well as chromosomes of the second meiotic division are often present in the reconstructed oocytes. Effects of the cell cycle of nuclear donor on the maturation of reconstituted GV oocytes have been analyzed (**Mohammed, 2006 a,b**). The study revealed that the S-phase blastomeres induce high maturation efficiency of reconstructed oocytes, but they simultaneously introduce chromosomal aberrations to almost all oocytes. Blastomeres in the G2 provide relatively high efficiency and low division distortions, but despite strict selection of the G2 phase, no more normally maturing oocytes have been obtained (**Mohammed, 2006 a,b**) than in the original attempts (**Grabarek *et al.*, 2004; Polanski *et al.*, 2005**). Moreover, **Polanski *et al.*, (2005**) showed that enucleation of the prophase oocyte results in decreased activity of Maturation Promoting Factor (MPF), and that different types of donor nuclei vary in their ability to restore normal levels of MPF and to support maturation. In addition, they found that transformation of cumulus cell nucleus into the pronucleus before transfer into the enucleated oocyte substantially improves meiotic maturation. Therefore, improvement maturation and developmental competence by selecting the nuclear donor and enriching the GV cytoplasts has been chosen as an alternative way. It is believed that nucleoli and nuclear sap supply the means to properly reprogram donor nuclei after they are introduced by nuclear transfer. Our wok (**Mohammed *et al.*, 2008**) show that selective enucleation of GV oocyte does not improve their maturation ability as compared with complete enucleation, after reconstruction with embryonic or somatic nuclei. Worthwhile, this is the extrusion of the first polar body from selective GV cytoplasts, but not from complete GV cytoplasts, reconstructed with GV nuclei which occur at exactly the same time as in the control oocytes, confirming the idea that GV-associated factors control the timing of the first meiotic division (**Polanski *et al.*, 1998**). Nucleoli do not appear to be involved in timing control, since enucleolated

GV oocytes extruded the first PB at the same time as did control oocytes (**Mohammed, unpublished observation**).

3.3. Stem cells

There are two major types of stem cells, according to the nitrogen bonds that held down the DNA, called "embryonic stem cells with no nitrogen bonds" and "adult or somatic stem cells with nitrogen bonds binding down some of their DNA". The DNA in any one cell is held down by nitrogen bonds, so the proteins, and therefore the body parts, they encode are not produced.

Embryonic stem cells are produced from embryos. An embryo is a group of cells produced immediately after fertilization. First, the zygote splits into two cells embryo, and then these split into more cells. This goes on through several phases, known as the gastrula phase and the blastula phase. At the gastrula phase and before, the cells aren't specified and specialized enough yet to have nitrogen bonds. Therefore, these cells are stem cells, and, since they come from an embryo, they are called embryonic stem cells.

An adult or somatic stem cell is thought to be an undifferentiated cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The problem with adult stem cells is that they are somewhat specialized and specified - they have nitrogen bonds binding down some of their DNA, and, therefore, cannot become any other type of cell - they can just become a range of cells, versus embryonic stem cells, which are true stem cells and which can become any other cell. When stem or somatic cells were introduced into matured GV cytoplasts, they formed abnormal pronuclei characterized by absence of membrane and nucleoli and they did not cleave further (**Gao *et al.*, 2002; Chang *et al.*, 2004; Mohammed *et al.*, 2008**).

4. Factors affecting maturation and developmental competence of reconstituted oocytes

The meiotic maturation of the reconstituted occytes and their developmental competence after activation or IVF strongly were affected by the type of donor nuclei and their cycle stages as well as the type of recipient cytoplasts as indicated previously. It has been indicated that the maternal material present in germinal vesicle is probably essential for proper formation of pronuclei and also for further development. It seems that nucleolar material itself may play an important role in formation of properly built pronuclei in addition to the helper role of cumulus cells on developmental potential of reconstituted oocytes. Nucleolus is the first intracellular structure components described. It has been accepted for a long time that the nucleolus is only a site of ribosomal RNA synthesis and maturation of RNA polymerase III transcripts, but its role is more complex (**Carmo-Fonseca *et al.*, 2000**).

4.1. Cumulus cells

Importance of cumulus cells for development of oocytes was indicated. Cumulus-enclosed oocytes resume meiosis when cultured in medium containing only glucose as a source of energy whereas cumulus-free oocytes do not. Nevertheless, addition of pyruvate to the oocyte culture medium provides support for the resumption of meiosis by denuded oocytes (**Donahue and Stern 1968**). This evidence indicates that cumulus cells take up and metabolize glucose to products that can be used by oocytes for the energy metabolism necessary to support meiotic maturation (**Leese and Barton, 1985**). Metabolic cooperatives of oocytes by cumulus cells involve the uptake of some amino acids, such as L-alanine, which are poorly transported into mouse oocytes and require uptake first by cumulus cells and then transfer to the oocyte via gap junctions (**Colonna and Mangia, 1983**). When oocytes are cultured with radiolabeled L-alanine, the amount of radioactivity detected in the cumulus-enclosed oocytes is greater than in cumulus-free oocytes (**Colonna and Mangia, 1983**). Blocking the function of gap junctions in cumulus enclosed oocytes abrogates this difference (**Haghighat and Van Winkle, 1990**). Recent study showed that oocytes control glycolysis in granulosa cells by regulating expression levels of genes encoding glycolytic enzymes (**Sugiura *et al.*, 2005**). Therefore, oocytes promote a key metabolic function of cumulus cells that is necessary for oocyte meiotic maturation and further embryonic development. Moreover, coculture with cumulus cells may assist the oocyte to avoid undergoing DNA fragmentation (**Wongsrikeao *et al.*, 2005**). Moreover, studies (**Yamazaki *et al.*, (2001; Fatehi *et al.*, 2002; Mohammed 2008)** found that the presence of loose cumulus cells partially restored the effect of denudation prior to in vitro fertilization. Collectively, the previous results suggest that cumulus-derived factors influencing cytoplasmic maturation reach the oocytes both through the gap junction coupling pathway and by diffusion.

The reported results by **Mohammed *et al.*, (2005)** indicated that addition of bovine follicular fluid (partially secreted from cumulus cells) to the maturation medium of bovine oocytes delayed timings of first cleavages in comparison of fetal calf serum. Our recent work (**Mohammed *et al.*, 2008)** showed that developmental competence of cumulus-enclosed cytoplasts was significantly increased compared with cumulus-free cytoplasts upon transfer of early embryonic nuclei.

4.2. The nucleolus assembly during oocyte growth and maturation stages

Changes in nucleolar morphology and transcriptional activity during the growth phase were described. At least in cattle, a functional fibrillogranular nucleolus is formed in the oocyte of early secondary follicles (**Fair *et al.*, 1997**). Transcriptionally active fibrillogranular nucleolus found in bovine growing oocytes is inactivated at an oocyte diameter of 110 µm, corresponding to an antral follicular diameter of approximately 3 mm (**Fair *et al.*, 1996, Fair *et al.*, 1997**). Therefore, a functional and transcriptional ribosome-synthesizing nucleolus is lacking from the fully grown oocyte stage of development and so-called nucleolar precursor bodies (NPBs), throughout the

two reduction divisions of meiosis. The nucleolus is formed as a compact fibrillar mass (**Zatsepina *et al.*, 2003**).

On the basis of the nucleolus– chromatin association, two distinct groups of fully grown oocytes can be collected from large antral follicles. In the first group, the nucleolus is surrounded by a heavily condensed chromatin, whereas in the second group, the chromatin is dispersed in the germinal vesicle nucleus of oocytes. These two groups of oocytes differ in the speed and extent of maturation where oocytes from the first group are more developmentally competent (**Combelles *et al.*, 2002; Miyara *et al.*, 2003**). The precise role of nucleolus during maturation is unkown. It has been indicated that nucleolus can be removed microsurgically (**Fulka *et al.*, 2003; Mohammed *et al.*, 2008**) from GV oocytes. Majority of enucleolated GV ocytes extruded first PBs during meiotic maturation (**Fulka *et al.*, 2003; Ogushi *et al.*, 2005; Mohammed *et al.*, 2008**) and show that a nucleolus is dispensable for meiotic maturation. Interestingly, when growing oocytes, which are still unable to mature, were enucleolated, germinal vesicle breakdown and chromosome condensation could be observed, but these oocytes never completed maturation and were arrested in metaphase I–like stage (**Fulka, unpublished cited in Fulka *et al.*, 2004)**.

4.3. The nucleolus assembly during zygote and embryo stages

Upon meiosis, nucleolus precursor bodies (NPBs) is established in the pronuclei. These entities harbour the development of fibrillogranular nucleoli and re-establishment of nucleolar function in conjunction with the major activation of the embryonic genome. This process referred to as nucleologenesis.

Upon enucleolation of GV oocytes, the resulting zygotes and embryos had pronuclei and blastomere nuclei respectively without visible nucleoli (after activation— Fulka, unpublished, cited in **Fulka *et al.*, 2004** after in vitro fertilization**— Ogushi *et al.*, 2005; Mohammed *et al.*, 2008; Fulka et al., 2011**).

Upon nuclear transfer, activation of complete GV cytoplasts reconstructed with somatic cells resulted in the formation of pronuclei without visible nucleoli (**Gao *et al.*, 2002; Chang *et al.*, 2004; Mohammed *et al.*, 2008**), contrary to pronuclei with visible nucleoli formed in those reconstructed with 1/2 and 1/8 blastomere nuclei. Our results (**Mohammed *et al.*, 2008**) indicated that GV nucleolus can be substituted by a nucleolus derived from embryonic cells (2-cell and, partially, 8-cell stage blastomeres), but not from somatic cells. In mammals, maternally derived transcripts and proteins are gradually degraded during early cleavage. At the same time nucleologenesis is observed: fibrillogranular nucleoli develop in the mouse at four-cell stage (**Geuskens and Alexandre, 1984**), although the first fibrillar centers are observed at the two-cell stage. Therefore, one cannot exclude that nucleoli in 1/8-blastomeres, but not in somatic fetal fibroblasts, may still contain some transcripts and polypeptides of maternal origin that can presumably be re-used to form a nucleolus. Conclusively, it appears that nucleologenesis in the embryo is based partly on the re-use of maternal transcripts and proteins inherited from the oocyte and partly on de novo proteins synthesized from the embryonic genome.

4.4. Nucleolus function and dysfunction during embryo development

Early development of the mammalian embryo is regulated by gene transcripts and polypeptides produced by, and stored in, the oocyte (nucles?) (**Schultz, 1993**). It is commonly accepted that a nucleolus is a specialized domain of the nucleus in which the production of rRNA and synthesis of ribosomes takes place. Some proteins with no known or clear relationship to ribosome biogenesis (**Scherl *et al.*, 2002; Politz *et al.*, 2005**) were described.

During in vitro production of embryos (**Niemann *et al.*, 2002**) and cloning by nuclear transfer (**Daniels *et al.*, 2000; Wrenzycki *et al.*, 2001**), initial embryonic development depends on gene expression is evidently disturbed. In vitro production system impairs nucleolgenesis in pig embryos whereas does not have any impact on nucleologenesis in bovine with respect to ultrastructure and allocation of nucleolar proteins. These findings are in accordance with higher developmental competence of in vitro-produced bovine embryos compared with their porcine counterparts.

The disorganization of nucleoli has catastrophic consequences for further embryonic development (Burns *et al.*, 2003; Zhang et al., 2013). Those investigators produced nucleoplasmin 2 (NPM 2)– deficient mice. It has been shown before in Xenopus laevis that nucleoplasmin is an oocyte-specific nuclear protein that removes protamines from sperm heads after fertilization and facilitates nucleosome assembly and paternal genome replication (**Hiyoshi *et al.*, 1991; Leno *et al.*, 1996**). Whereas NPM 2_/_ mouse males were normal and fertile, NPM 2_/_ mouse females had severe fertility defects. The detailed analysis of oocyte maturation and early embryonic development in these females showed the disruption of nucleoli in oocytes and early embryos. This influences some vital processes that are important for normal development, that is, hypoacetylation of histone H3 and ribosomal RNA synthesis. Interestingly, the decondensation of sperm head and the formation of both (male and female) pronuclei occur normally, but nucleoli could not be detected in them. In the light of these results, the nucleolar dysfunction in oocytes and also in embryos could be the cause of infertility. This has been also supported by observations when the size and morphology of pronuclei in human zygotes were evaluated. Not only the size of them, but also their position and number, size, and location of nucleoli reflected the developmental ability of embryos (**Tesarik and Greco 1999; Tesarik *et al.*, 2000; Gianaroli et., 2003**). Moreover, as previously mentioned upon enucleolation of GV oocytes, the resulting zygotes and embryos had pronuclei and nuclei characterized by absence of nucleoli. Majority of the

resulting embryos were mostly blocked after the first cleavage.

Upon nuclear transfer, developmental competence of both activated and fertilized GV oocytes reconstructed with embryonic or somatic nuclei was limited to the zygote stage. The absence of nucleoli (as previously discussed) in the pronuclei after activation (**Gao etal., 2002; Chang *et al.*, 2004; Mohammed *et al.*, 2008**) and/or impaired donor pronuclei formation after IVF (**Mohammed *et al.*, 2008**) might contribute to this developmental block.

The direct cause of cleavage inhibition might be the misalignment of chromosomes in metaphase spindle **(Chang *et al.*, 2004; Polanski *et al.*, 2005; Mohammed *et al.*, 2008)**. The very long first meiotic M phase in the oocyte (**Polanski, 1986, Polanski 1997**) might be needed to assure (i) proper positioning of the meiotic spindle for asymmetric division (**Maro and Verlhac, 2002**), (ii) maintenance of bivalents to prevent sister chromatid separation during the first meiosis also by suppression of biorientation of sister kinetochores (**Petronczki *et al.*, 2003**), and (iii) controlled biphasic destruction of cohesins (**Kitajima *et al.*, 2004**). Inability by oocytes reconstructed with donor nuclei to meet these requirements can adversely affect their first cleavage. Therefore, selective enucleation and introducing the donor nuclei after maturation of cytoplasts are applied to overcome absence of nucleoli and misalignment of chromosomes in metaphase spindle.

When 1/2-blastomere nuclei were introduced into matured cytoplasts, both the nuclear contents which were released into the cytoplasm with selective enucleation and the presence of cumulus cells during cytoplasts' maturation increased significantly the cleavage rate of activated as well as the percentage of embryos which were able to develop till blastocyst stage. Although activated reconstructed selective GV cytoplasts were also cleaved and few of them developed to the blastocyst stage the frequency of cleavage to the 4-cell stage and blastocyst formation was evidently lower than those of reconstructed selective GV cytoplasts cumulus enclosed. Also, the mean cell number in the obtained blastocysts was slightly higher in blastocysts derived from activated reconstructed selective GV cytoplasts cumulus enclosed **(Mohammed *et al.*, 2008)**.

The studies indicate that it is possible to obtain full preimplantation development after transfer of early embryonic nuclei into selective GV cytoplasts. However, the presence of GV matarial in oocyte' cytoplasm as well as the presence of cumulus cell during maturation seem to be conditions *sine qua non* of successful development.

5. Summary and future prospects

Creation of artificial gametes has been recently proposed as the ultimate solution in the treatment of infertility or embryonic/somatic cloning. Nuclear transfer studies have demonstrated that maturation (haploidization) is feasible of germ cells, whereas it is limited of embryonic and somatic cells, under certain experimental conditions. Additionally, activation and fertilization was also achieved using haploidized germ, embryonic or somatic cells in experimental conditions. Correct segregation of germ chromosomes takes place whereas embryonic or somatic chromosomes does not take place in the ooplasts and this was identified as the primary cause of failure of the embryonic or somatic haploidization and their further developmental competence.

More recently a new approach using selective enucleation as an alternative to complete enucleation has shown promise. Selective GV cytoplasts upon reconstruction with GV nuclei restore delayed of timing polar body extrusion and probably proper pronuclei formation and further developmental competence compared with complete GV cytoplasts. The developmental competence was significantly increased with the presence of cumulus cells around the oocytes.

Germinal vesicle transfer into selective GV cytoplasts increases the nuclear materials in the cytoplasts to the double. It is expected that such cytoplasts upon reconstruction with embryonic or somatic nuclei may increase (i) maturation, fertilization and further developmental competence (ii) rescue developmental incompetency of oocytes nuclear or cytoplasmic in nature (iii) reprogramming of germ, embryonic or somatic donor nuclei through proper pronuclei formation and increasing the cell number of the resulting embryos.

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